Molecular Plant • Volume 5 • Number 2 • Pages 304–317 • March 2012 REVIEW ARTICLE

Deciphering the Enigma of Lignification: Precursor Transport, Oxidation, and the Topochemistry of Lignin Assembly

Chang-Jun Liu[1]

Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA

ABSTRACT Plant lignification is a tightly regulated complex cellular process that occurs via three sequential steps: the synthesis of monolignols within the cytosol; the transport of monomeric precursors across plasma membrane; and the oxidative polymerization of monolignols to form lignin macromolecules within the cell wall. Although we have a reasonable understanding of monolignol biosynthesis, many aspects of lignin assembly remain elusive. These include the precursors' transport and oxidation, and the initiation of lignin polymerization. This review describes our current knowledge of the molecular mechanisms underlying monolignol transport and oxidation, discusses the intriguing yet least-understood aspects of lignin assembly, and highlights the technologies potentially aiding in clarifying the enigma of plant lignification.

Key words: lignification; monolignol transport; ABC transporter; laccase; peroxidase.

INTRODUCTION

Lignin is complex phenypropanoid polymer derived primarily from three cinnamyl alcohols: *p*-coumaryl, coniferyl, and sinapyl alcohols (termed monolignols). It is a crucial structural component preserving the integrity of plant cell wall, imparting stiffness and strength of vascular plants, enabling the transport of water and solutes through the tracheary elements in the vasculature system, and affording physical barriers against invasions of phytopathogens, and other environmental stresses (Boerjan et al., 2003; Ralph et al., 2004a). However, its presence contributes to the recalcitrance of cell wall to degradation, and thus is detrimental to using cellulosic fibers in cattle feedstock, for pulping and paper making, and for producing liquid biofuels (Chen and Dixon, 2007; Li et al., 2008; Weng et al., 2008).

Plant lignification is a cellular process generating lignin polymer in the cell wall. In general, it occurs in three stages: the biosynthesis of monolignols in the cytosol; the transport of these monolignols to the cell wall; and their subsequent oxidative dehydrogenation and polymerization to form heterogeneous macromolecules. Over several decades, extensive studies have centered on monolignol biosynthesis, particularly on elucidating its pathways and the molecular regulation of the biosynthetic processes. The progresses have been intensively reviewed correspondingly (Anterola and Lewis, 2002; Boerjan et al., 2003; Ralph et al., 2004a; Ralph, 2007; Li et al., 2008; Vanholme et al., 2008; Zhong and Ye, 2009; Umezawa, 2010; Vanholme et al., 2010; Zhao and Dixon, 2011). Compared to the knowledge of monolignol biosynthesis, our understanding of lignin assembly, namely the transport of lignin precursors, their deposition, and subsequent activation and polymerization, is fragmentary. In this review, we outline the different speculations on the sequestration, transport, and subsequent spatial deposition of the monolignols, and describe recent progresses in molecular genetics and biochemical studies aimed at gaining an understanding of the underlying molecular mechanisms for translocating monolignols across cell membranes, advances in identifying the oxidative dehydrogenation enzymes, and progresses and issues in detailing the topochemistry of lignification. Meanwhile, we highlight the technological developments that might be applied productively to explore lignin assembly.

[1] To whom correspondence should be addressed. E-mail cliu@bnl.gov, tel. 631-344-2966, fax 631-344-3407

doi: 10.1093/mp/ssr121, Advance Access publication 3 February 2012
Received 29 September 2011; accepted 13 December 2011

MONOLIGNOL BIOSYNTHESIS AND THE POTENTIAL PHYSICAL ORGANIZATION OF THE ENZYMES

Originating from the shikimate pathway, wherein plant produces aromatic amino acids, the biosynthesis of monolignols starts from the deamination of phenylalanine by the entry point enzyme phenylalanine ammonia lyase (PAL), and then undergoes subsequent aromatic-ring modifications via hydroxylation and methylation, and the transformation of the carboxylic moiety of the propane tail through esterification and reduction. This process yields primarily three hydroxycinnamoyl alcohols, *p*-coumaryl, coniferyl, and sinapyl alcohols (i.e. monolignols) (Umezawa, 2010; Vanholme et al., 2010). More than 10 enzymes sequentially catalyze this synthetic pathway (Figure 1). Three of them, namely cinnamic acid 4-hydroxylase (C4H), *p*-coumaroylshikimate 3′-hydroxylase (C3′H), and coniferaldehyde/ferulic acid 5-hydroxylase (F5H), are membrane-anchored cytochrome P450 proteins that predictably associate with the outer surface of the endoplasmic reticulum (ER) by virtue of their N-terminal membrane anchor (Li et al., 2008). Nevertheless, many other enzymes, such as phenylalanine ammonia lyase (PAL), 4-hydroxycinnamoyl CoA ligase (4CL), caffeoyl CoA *O*-methyltransferase (CCoAOMT), hydroxycinnamoyl CoA reductase (CCR), caffeic acid/5-hydroxyferulic acid 3/5-*O*-methyltransferase (COMT), and (hydroxy)cinnamyl alcohol dehydrogenase (CAD) found in diverse species operationally are soluble proteins and likely to be located within the cytosol (Takabe et al., 1985; Nakashima et al., 1997; Chen et al., 2000). Apparently, the different compartmental propensity of the monolignol biosynthetic enzymes, together with the exclusive plastidic localization of the shikimate pathway that leads to the synthesis of aromatic amino acids for phenylpropanoids (Herrmann and Weaver, 1999; Rippert et al., 2009), implies either the occurrence of multiple subcellular sequestration of metabolic intermediates or the ideal physical organization of

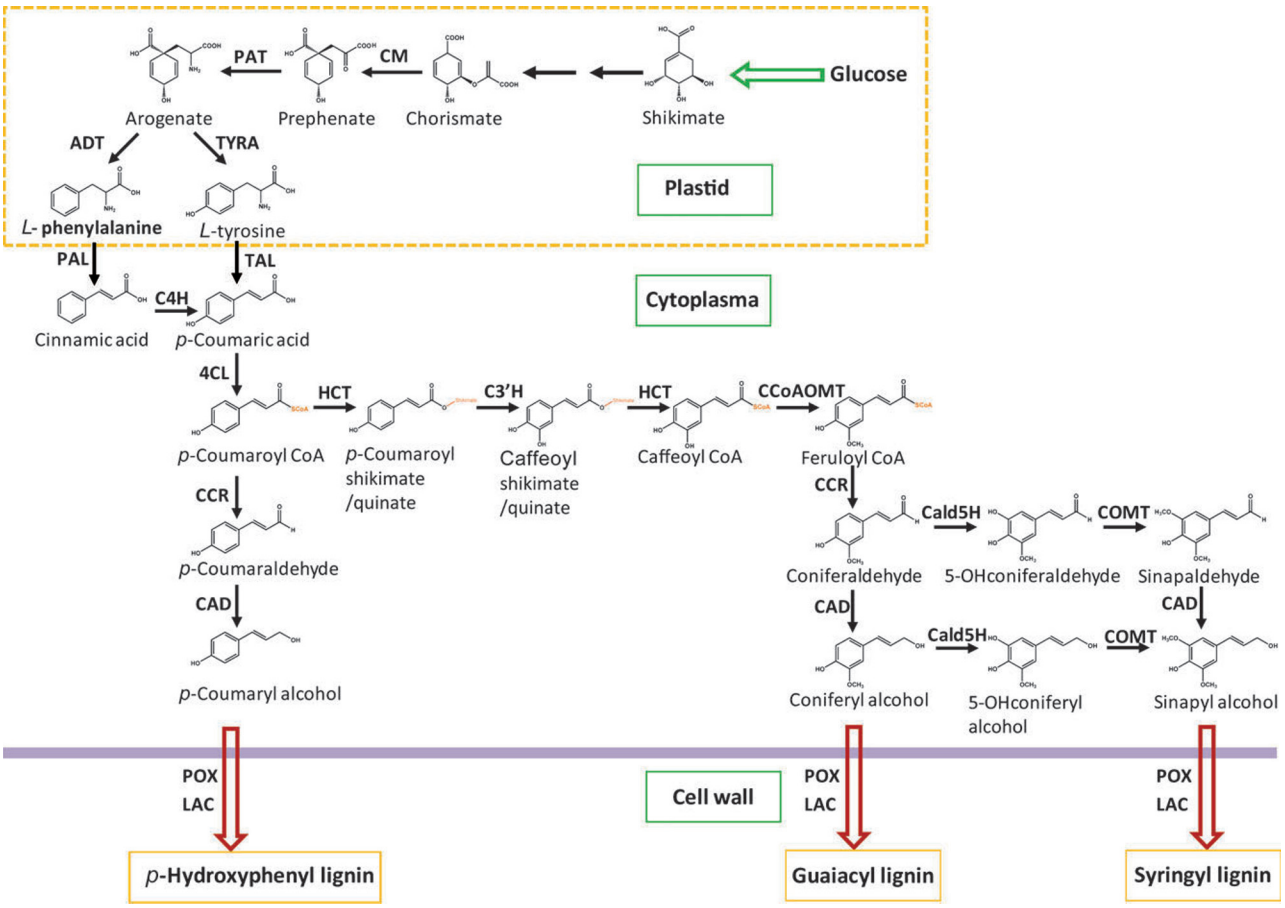

Figure 1. The Scheme of the Simplified Shikimate–Phenylpropanoid–Lignin Biosynthetic Pathway, Illustrating Different Compartmentalization of the Biosynthesis.

CM, chorismate mutase; TYRA, arogenage dehydrogenase; ADT, arogenate dehydratase; PAT, prephenate aminotransferase; PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-hydroxycinnamoyl CoA ligase; HCT, hydroxycinnamoyl; CoA, shikimate/quinate hydroxycinnamoyltransferase; C3′H, *p*-coumaroylshikimate 3′-hydroxylase; CCoAOMT, caffeoyl CoA *O*-methyltransferase; CCR, cinnamoyl CoA reductase; Cald5H/F5H, coniferaldehyde/ferulate 5-hydroxylase; COMT, caffeic acid/5-hydroxyferulic acid *O*-methyltransferase; CAD, (hydroxy)cinnamyl alcohol dehydrogenase; POX, peroxidase; LAC, laccase.